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Peanut oral immunotherapy increases IgG4 to Ara h 1, 2, and 6 but does not affect IgG4 to other allergens

Short title: Peanut oral immunotherapy increases IgG4 to Ara h 1, 2, and 6

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To the Editor:

Life-threatening allergic reactions are characterized by allergen-specific IgE antibodies, whereas IgG antibodies are a default response to an antigen exposure. Subclass 4 IgG antibodies function as blocking antibodies, which may suppress IgE-mediated reactions.¹ Peanut oral immunotherapy (OIT), as other allergen immunotherapies, induces IgG4 antibodies.^{2,3} We have previously shown the increase of IgG4 to peanut seed storage proteins, and the dose-dependent increase of IgG4- IgE ratios in children and adolescents receiving peanut OIT.^{4,5} Further, we showed that IgE to the 2S albumins Ara h 2 and Ara h 6, which are the most important peanut allergens,⁶ were the only IgE responses affected during OIT.⁵

In this study, we screened IgG4 responses for a wide spectrum of allergens with the ISAC ImmunoCAP microarray in children and adolescents who underwent peanut OIT. We examined the IgG4 responses baseline and after OIT build-up phase with the aim of finding effects of the treatment on IgG4 responses to peanut-specific and cross-reactive allergens.

The study population has been described in our previous publications.^{4,5} Briefly, 6- to 18-year-old children and adolescents were referred to the hospital because of peanut allergy. Of them, 39 with a moderate-to-severe reaction in a double-blind placebo-controlled peanut

challenge started OIT and 21 control patients continued to avoid peanut. During OIT, the daily dose of peanut protein increased from 0.1 to 800 mg in eight months. Serum samples were drawn at baseline and after median 12 months of OIT build-up phase or avoidance. We analyzed serum IgG4 (30 µl of sample) in ImmunoCAP ISAC microarrays (ThermoFisher Scientific; Uppsala, Sweden). We considered the detection limit 0.1 ISU-G4 also as cut-off for positive response. The ethics committee of Helsinki University Hospital approved the study. One of the parents and the patient gave a written informed consent.

In analyzing repeated measures of the 112 allergens, we filtered the results so that in order to be included in the analysis an allergen had to show value above detection limit 0.1 (ISU-G4) in at least 50% + 1 of the patients in at least one of the four groups (pre OIT, post OIT, pre avoidance, post avoidance). We fitted a linear model to investigate the effects of treatment, response, subject, sex, and age. Furthermore, we used the eBayes test for pairwise comparisons of interest. P values were corrected in each pairwise comparison by using the Benjamini-Hochberg procedure. We used R program, version 3.0 (R Project for Statistical Computing, R Foundation, Vienna, Austria; <http://www.r-project.org/>) and IBM SPSS 22 (IBM, Armonk, NY).

Of the 39 patients on OIT, 34 achieved maintenance dosing, and 26/31 passed the double-blind placebo-controlled challenge with 1255 mg peanut protein. Baseline characteristics and ISAC IgE levels are presented in the online supplemental table 1. IgG4 levels to Ara h 1, 2, and 6 increased during the treatment. Avoidance group showed no changes in IgG4 levels. (Figure 1) The ingested cumulative protein dose during the treatment was median 71 g (range 50 mg to 158 g). The cumulative protein dose correlated with the increase in IgG4 to Ara h 2 (ρ 0.362, P = .024), but did not correlate with the IgG4 increase of

Ara h 1 and Ara h 6.

The IgG4-increases for Ara h 1, 2, and 6 in the 26 patients who passed the final peanut challenge, i.e. desensitized patients, did not differ statistically from the five non-desensitized. (Data not shown)

Levels of other than peanut allergens that showed representative numbers of response are depicted in table 1, and other ISAC112 allergens in the online supplemental table 2.

To our knowledge this is the first study to examine microarrayed IgG4 profiles in patients who received peanut oral immunotherapy. During the treatment, the only IgG4 levels to change were peanut seed storage proteins Ara h 1, 2, and 6. This is in line with our previous study where peanut OIT affected IgE only for the 2S albumins Ara h 2 and 6. However, in the IgG4 antibodies, the effect included also a third seed storage protein, Ara h 1. Studies on peanut sensitization and allergy show that the 7S globulin Ara h 1 is more associated with peanut allergy than the 11S globulin Ara h 3.^{7,8} In our previous study, IgG4, measured with ImmunoCAP, showed increases in Ara h 1 and Ara h 3, of which Ara h 1-change was stronger.⁵ In the present study, Ara h 3-response in IgG4 ISAC was underrepresented and therefore conclusions on effects on this allergen are limited. However, the response rate increased from 15% before OIT to 44% after OIT. (Table 1 b) We believe that mild increase in Ara h 3 IgG4 in ISAC is possible.

The findings of unaffected Ara h 8- and Ara h 9- IgG4-levels are similar to our previous study on IgE microarray profiles in this patient population.⁵ Ara h 8 is degraded in the gastric digestion and its' effects on IgG4 levels may be therefore limited. The amount of lipid transfer protein Ara h 9 is low in peanut⁹ which in turn may decrease the immunological effects of this stable allergen. Both Ara h 8- and 9-IgG4 responses were infrequent in our study population.

Considering the development of tolerance, IgG4 may be the most important immunoglobulin in immunotherapy. Findings of this study further highlight the specificity of immunological response in peanut oral immunotherapy as IgG4 effects seem to occur only on the most peanut-specific allergens, but not on cross-reactive or unrelated allergens.

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ABBREVIATIONS

Immuno-Solid phase Allergen Chip (ISAC)

ISAC standardized units (ISU)

Oral immunotherapy (OIT)

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a)

OIT	Pre			Post			P value	Pre	Post
	Median	Min	Max	Median	Min	Max		No of patients with response	
Bet v 1	0.32	0.05	3.6	0.46	0.05	3.1	.65	30	31
Mal d 1	0.10	0.05	2.4	0.12	0.05	2.1	.85	20	22
Bos d 8	0.50	0.05	11.4	2.3	0.05	22.4	.85	28	30
Bos d 5	2.1	0.05	17.6	2.4	0.05	18.8	.85	36	36
Bos d 4	2.1	0.05	21.6	0.48	0.05	13.7	.85	28	27
Bos d Lactoferrin	0.56	0.05	5.9	0.50	0.05	8.0	.85	28	30
Gal d 1	0.88	0.05	19.6	1.1	0.05	22.6	.85	32	34
Gal d 2	1.8	0.05	13.3	2.2	0.05	11.9	.85	33	32
Gal d 3	0.12	0.05	0.80	0.14	0.05	0.70	.85	21	25
Tri a aA_TI	0.28	0.05	2.9	0.26	0.05	3.2	.85	25	26
Tri a 14	0.05	0.05	9.5	0.10	0.05	14.4	.85	19	21
Avoidance	Pre			Post			P value	Pre	Post
	Median	Min	Max	Median	Min	Max		No of patients with response	
Bet v 1	0.26	0.05	14.1	0.16	0.05	0.90	1	15	12
Mal d 1	0.12	0.05	2.4	0.12	0.05	1.0	1	11	10
Bos d 8	0.74	0.05	31.0	0.32	0.05	27.9	1	19	17
Bos d 5	3.7	0.05	25.1	2.0	0.05	19.2	1	19	17
Bos d 4	3.7	0.05	28.9	2.7	0.05	23.5	1	19	17
Bos d Lactoferrin	0.47	0.05	1.9	0.55	0.05	1.8	1	17	15
Gal d 1	1.5	0.05	13.6	1.6	0.05	18.1	1	18	16
Gal d 2	3.7	0.05	19.8	2.5	0.05	24.4	1	19	17
Gal d 3	0.24	0.05	1.2	0.16	0.05	1.4	1	16	14
Tri a aA_TI	0.20	0.05	2.4	0.14	0.05	6.4	1	16	14
Tri a 14	0.18	0.05	5.7	0.05	0.05	26.2	1	14	8

b)

OIT			Avoidance		
	Pre, n (%)	Post, n (%)		Pre, n (%)	Post, n (%)
Ara h 1	7 (18)	27 (69)		8 (44)	6 (33)
Ara h 2	15 (38)	37 (95)		11 (61)	7 (39)
Ara h 3	6 (15)	17 (44)		5 (28)	3 (17)
Ara h 6	16 (41)	37 (95)		11 (61)	10 (56)
Ara h 8	7 (18)	10 (26)		2 (11)	0
Ara h 9	12 (31)	12 (31)		3 (17)	4 (22)

Table 1. a) IgG4 levels and number of patients with response ($\text{ISU-G4} \geq 0.1$) before and after treatment in oral immunotherapy (OIT) (n=39) and avoidance (n=18) groups. Allergens that showed representative response are included. (see methods section) **b)** Number of patients with IgG4 response ($\text{ISU-G4} \geq 0.1$) for peanut allergens in immunotherapy and avoidance groups.

Figure and supplementary table legends:

Figure 1. Levels of IgG4 to peanut allergens in a) oral immunotherapy group (n=39) and b) avoidance group (n=18) before and after treatment. Ara h 3, Ara h 8, and Ara h 9 are excluded from the statistical analysis due to limited number of responses. (see Methods section)

Supplemental table online 1. Baseline characteristics of patients in OIT (n=39) and avoidance (n=18) groups.

Supplemental table online 2. Levels of IgG4 and number of patients with response (ISU-G4 ≥ 0.1) before and after peanut oral immunotherapy. Allergens with no response in any of the patients are excluded from the table (Amb a 1, Ani s 1, Ani s 3, Api m 1, Art v 1, Asp f 3, Asp f 6, Ber e 1, Bet v 4, Bla g 7, Blo t 5, Che a 1, Cla h 8, Der f 2, Der p 1, Der p 2, Fag e 2, Gal d 5, Hev b 1, Hev b 3, Hev b 5, Hev b 6.01, Ole e 1, Pen m 2, Phl p 11, Phl p 12, Phl p 2, Phl p 7, Pla a 1, Pla l 1, Pol d 5, Tri a 19.0101, and Ves v 5.).

